Breaking Tolerance—Another Piece Added to the Vitiligo Puzzle

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Vitiligo presents with striking depigmented cutaneous lesions and a histologic loss of functional melanocytes and melanin. Three major theories exist to explain this loss of melanocytes. First, the destruction of melanocytes is thought to be mediated by external factors including changes in neurological mediators that affect melanocyte function and/or survival. Second, melanocytes are altered by intrinsic factors including toxic metabolites within the melanin biosynthesis pathway (Lerner, 1971) that affect structure and/or function. Third, an autoimmune mechanism leads to melanocyte destruction.

Autoimmune Vitiligo

The evidence for autoimmunity is based on multiple clinical and research findings in humans and animals (Ongenae *et al*, 2003). Vitiligo is associated with coexisting autoimmune disorders and organ-specific antibodies. In addition, routine non-surgical repigmenting treatment utilizes immune-modulators including topical corticosteroids and inhibitors of the calcineurin pathway (tacrolimus). These indirectly support the argument for an immune etiology of disease. Myeloid cells are also implicated in that monocytes from patients with active disease produce increased proinflammatory cytokines and CD68+ macrophages are abundant in the dermis. Large patient groups have also shown aberrations in T cell and natural killer cell profiles in vitiligo patients supporting a role for cell-mediated immunity. T cell infiltrates are invariably seen at the site of depigmentation in patients with active disease. Furthermore, high frequencies of cytotoxic T lymphocytes specific for melanocytic antigens are detected in vitiligo patients, implicating a direct melanocyte specific T cell attack.

Autoimmunity and Tolerance

The hallmark of organ-specific autoimmune diseases is the disruption of the immune system's normal homeostatic balance leading to tissue destruction. In healthy people the immune system maintains the ability to recognize 25 million different antigens (Arstila *et al*, 1999) and provides surveillance against a multitude of foreign pathogens without any pathologic response to self-antigens. There are two known mechanisms by which we become tolerant or unresponsive to self-antigens. First, T and B cells are deleted in the thymus and bone marrow to remove those cells that pose the greatest threat. Despite this mechanism (known as central deletion or central tolerance), some precursor self-reactive cells will escape deletion, mature, and populate peripheral tissues but still do not attack self. The second mechanism of tolerance, a phenomenon known as peripheral tolerance, involves the regulation of self-reactive cells by multiple regulatory pathways. A central hypothesis in vitiligo research is that immune tolerance to self-melanocyte antigens is broken.

In this issue of the *Journal of Investigative Dermatology*, Steitz *et al* demonstrate two requirements to break tolerance to a MHC class I-restricted peptide derived from a melanocyte-derived self-antigen, murine tyrosinase-related protein 2 (TYRP2) (Tüting *et al*, 2004). The two requirements are (1) CD4 T cell help and (2) local inflammation at the site of self-antigen endogenous expression. Although previously the authors showed the repeated local intradermal immunization led to the local depigmentation of melanocytes, now the authors show that systemic intraperitoneal immunization must be accompanied by a subsequent local gene gun injection of plasmid DNA-coated gold particles or application of the contact allergen 2,4-dinitrofluorobenzene (DNFB). The data provide evidence that both CD4+ T cell help induced by the systemic immunization as well as local inflammation are required to break MHC class-I-restricted T cell tolerance.

The exact nature of the local inflammation required to break the tolerance remains, however, to be examined, and could be immunologically specific or non-specific in nature. One may extrapolate that the unmethylated CpG (CG-rich sequences, also referred to as immunostimulatory sequences, ISS) contained within the double stranded plasmid DNA produced by bacteria may stimulate the innate arm of the immune system by signaling through the pattern recognition receptor, Toll-like receptor 9 (TLR9), to provide a "danger signal" (Matzinger, 1994). TLR9 ligands have been shown to stimulate the production of interferon (IFN)-γ, IFN-α, IFN-β, and interleukins (IL) 12 and 18, all of which foster Th1 responses and enhance cell-mediated immunity (Roman *et al*, 1997). The requirement for TLR9 could also be tested by repeating these experiments in TLR9- or myeloid differentiation factor 88 (MyD88)-deficient mice. MyD88 is a downstream signaling intermediary important for the effects of TLR9 ligation. An alternative possibility is that the inflammation is nonspecific, caused by the mechanical trauma induced by the introduction of gold particles into skin. It would therefore be interesting to know whether gold particles without DNA would also trigger depigmentation.

Minor trauma alone is well known to cause new skin lesions in vitiligo patients. This is known as the Koebner phenomenon (Sweet, 1978), first described in psoriasis, which refers to the appearance of clinical lesions in "uninvolved skin" as a result of mechanical trauma. In psoriasis, this uninvolved skin is not, however, normal, but expresses a predominance of CD4 over CD8 T cells in the epidermis compared with skin from normal donors. It is hypothesized that these immune alterations in the presence of the trauma-induced alterations lead to clinical disease. Trauma in Koebner-prone psoriatic patients stimulates the accumulation of CD4+ T cells at the site of injury (Paukkonen *et al*, 1992). To our knowledge similar studies have not been performed in vitiligo patients.

There is much to learn about vitiligo from studies of melanoma vaccination in patients with metastatic disease undergoing immunotherapy. One vaccination strategy has been to enhance T cell activation during immunization by blocking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a critical receptor that downregulates T cell activation. Prior treatment with CTLA-4 before with the melanocyte-specific protein gp100 resulted in vitiligo in 2/14 patients (Phan *et al*, 2003). In other studies (Houghton, 1994), immunizations using xenogeneic orthologs to facilitate T cell recognition or heteroclitic epitopes with increased affinity to the MHC also results in vitiligo. These data indicate that normal regulatory pathways prevent the emergence of immune reactivity to self-antigens expressed by melanocytes, yet immune dysregulation can lead to vitiligo.

Breaking Tolerance—New Concepts

Recent immunologic insights into mechanisms of immune tolerance impact our understanding and potential treatment of vitiligo. It is now well known that T cells produce distinct patterns of cytokines that are cross-regulatory in nature; Th1 cytokine are involved in cell-mediated immunity and Th2 cytokines in humoral responses. These cytokine patterns influence the outcome of microbial infection in skin (Yamamura *et al*, 1991) and contribute to the pathogenesis of many inflammatory skin disorders. The skewing of cytokine responses from one pattern to another is termed immune deviation. Much experimental and clinical work is underway in the attempt to deviate the immune response to downregulate a destructive immune response. The goal of this therapy is to increase T helper cells that secrete a Th2 cytokine profile (IL-4, IL-5, IL-10, IL-13). This has been successfully demonstrated in experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis, where an antigen-specific Th1 response is pathogenic and a Th2 response is protective (Young *et al*, 2000). Both helper and cytotoxic T cells from progressing margins generate predominantly type 1 cytokines, namely, IFN-γ and TNF-α (Le Poole *et al*, 2004), suggesting vitiligo is a Th1-mediated disease and perhaps immunization to skew the immune response to a Th2 phenotype may prevent further progression.

An exciting new area of immunologic interest has been the identification of immune-regulatory cells (Tregs) and their role in autoimmune disease (Sakaguchi, 2004). Depletion or functional alteration of naturally occurring CD4+ Tregs, the majority of which express CD25, leads to the development of autoimmune disease in otherwise normal animals. Tregs cells are a functionally distinct and mature subpopulation of T cells. They possess a repertoire of antigen specificities as broad as that of naïve T cells, and are capable of recognizing both self- and non-self-antigens, thus enabling them to control various immune responses. Comparison of peripheral blood from patients with vitiligo *versus* healthy controls has revealed the increased expression of the activation-associated surface antigen CD25 (Mahmoud *et al*, 2002). The increased CD25 expression in vitiligo patients has been interpreted to reflect increased antigen-mediated activation, but it might also reflect an increase in Tregs. In an animal model of melanoma immunotherapy, depletion of all CD4+ T cells including Tregs resulted in enhanced B16 tumor rejection suggesting a role for Tregs in maintaining tolerance to melanocyte self-antigens (Nagai *et al*, 2000). Further studies are required to determine whether the dysregulation of Tregs is one of the factors that can break tolerance to melanocyte self-antigens and contribute to the pathogenesis of vitiligo.

By understanding the mechanisms that can break tolerance, it is possible to design rational strategies to restore tolerance in autoimmune disease. This could be achieved by one or a combination of different approaches (Steinman, 2004). One is the induction of anergy, whereby the pathogenic T cell is rendered unresponsive to self-antigen. Another strategy is to delete autoreactive T cells by inducing apoptosis, using a mechanism termed activation-induced cell death. A third attractive approach is by induction of Tregs specific for autoantigens targeted by pathogenic T cells. The ideal therapy would simultaneously delete pathogenic T cells and induce Tregs. Our ability to manipulate immune-regulatory pathways holds great promise for developing new treatments for vitiligo.

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